the burial of the solvent accessible surface area upon ligand binding slightly contributed to the binding energy. However, the polar solvation term produced unfavourable component to the overall binding free energy. The order of magnitude for the averaged binding free energy was TBHQ > TB2 > TB3, consistent with the experimental measurements.

Table 1. The MM/PBSA binding free energies and their energy contributions (in kcal/ mol) as well as the estimated experimental binding free energies for TBHQ, TB2 and TB3.

System	TBHQ			TB2			TB3		
	Site-1	Site-2	Site-3	Site-1	Site-2	Site-3	Site-1	Site-2	Site-3
$\langle \Delta E_{elec} \rangle_{AV}$	−19.07 (7.82)	−20.07 (3.35)	−22.79 (6.95)	−17.67 (4.00)	−25.69 (3.23)	−50.73 (4.92)	−5.86 (2.63)	−9.95 (6.26)	−7.65 (5.15)
$\langle\langle \Delta E_{elec} \rangle\rangle_{AV}$		−20.64			−31.36			−7.82	
$\langle \Delta E_{vdw} \rangle_{AV}$	−22.5 (3.70)	−18.75 (2.18)	−18.94 (2.46)	−21.7 (1.63)	−25.4 (1.72)	− 17.75 (2.00)	−17.57 (1.37)	−16.37 (1.9)	−15.57 (1.50)
$\langle\langle \Delta E_{vdw} \rangle\rangle_{AV}$		−20.06			−21.62			−16.50	
$\langle \Delta E_{MM} \rangle_{AV}$	−41.56 (6.25)	−38.82 (2.85)	−41.65 (6.13)	−39.37 (4.41)	−51.08 (3.74)	−68.47 (4.45)	−23.42 (2.87)	−26.32 (6.03)	−23.22 (5.07)
$\langle\langle \Delta E_{MM} \rangle\rangle_{AV}$		−40.68			−52.97			−24.32	
$\langle \Delta G^{0}_{nonpol} \rangle_{AV}$	−3.87 (0.74)	−3.80 (0.1)	−3.53 (0.17)	−3.25 (0.38)	−3.16 (0.11)	−2.95 (0.08)	−2.66 (0.08)	−2.63 (0.19)	−2.49 (0.4)
$\langle\langle \Delta G^{0}_{nonpol} \rangle\rangle_{AV}$		−3.72			−3.12			−2.59	
$\langle \Delta G^{0}_{pol} \rangle_{AV}$	36.03 (7.30)	36.29 (4.46)	34.03 (6.73)	47.96 (7.84)	51.02 (5.72)	59.67 (4.23)	21.22 (5.09)	23.74 (3.95)	22.06 (4.72)
$\langle\langle \Delta G^{0}_{pol} \rangle\rangle_{AV}$		34.45			58.88			22.34	
$\langle \Delta G^{0}_{sol} \rangle_{AV}$	32.17 (7.11)	32.48 (4.43)	30.50 (6.68)	44.71 (7.85)	47.87 (5.71)	56.72 (4.22)	18.56 (5.08)	21.11 (3.96)	19.57 (4.73)
$\langle\langle \Delta G^{0}_{sol} \rangle\rangle_{AV}$		31.72			50.10			19.75	
$\langle \Delta G^{0}_{bind} \rangle_{PBSA}$	−9.40 (5.36)	−6.34 (3.97)	−11.15 (3.97)	−5.33 (5.95)	−3.22 (4.36)	−11.75 (2.48)	−4.86 (4.34)	−5.21 (5.21)	−3.65 (4.07)
$\langle\langle \Delta G^{0}_{bind} \rangle\rangle_{PBSA}$		−8.96			−6.76			−4.57	
$\Delta G^{*}_{experiment}$		−7.2			−4.1			−4.1	

* Estimated from experimental IC_{50} (ΔG= RT ln IC_{50})

V. Conclusion

HA, playing a crucial role in the influenza viral replication cycle, has emerged as an attractive target. A small molecule TBHQ has been reported to prevent the HA conformational change, thereby inhibiting HA-mediated entry and presenting a potential avenue for novel antiviral interventions. Here, MD simulations of H3N2 HA complexed with TBHQ, and its derivatives (TB2 and TB3) were conducted. TBHQ demonstrated a higher number and stronger hydrogen bond interactions with the HA virus compared to its analogue compounds. Both hydrophobic and hydrogen bonding interactions are the key elements to stabilize HA and its fusion inhibitors. Our hypothesis posits that compounds capable of forming hydrogen bonds with both $E57_1$ of one HA protomer and $E97_2$ of another protomer may effectively stabilize the neutral pH structure of HA. This stabilization, in turn, would prevent the conformational rearrangement necessary for membrane fusion to occur.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Nadtanet Nunthaboot conducted the research and wrote the paper. Thitiya Boonma analyzed the data. Chananya Rajchakom produced figures and graphics. All authors had approved the final version.

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